THE EFFECT OF INORGANIC TRIPHOSPHATE ON THE INTERACTION OF Ni (II) WITH ADENOSINE

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1. Introduction

It is well documented that a number of divalent metal ions can interact with the purine base of ATP but only poorly or not at all with the same purine base in adenosine [1]. This is particularly true in the case of transition metals such as Co(II), Mn(II) and Ni(II). There are two possible explanations of this phenomenon. These are: (a) because of the three dimensional structure of the nucleotide the metal complexed to the phosphates is held in a position that favors its interactions with the purine base; and (b) the interaction of the metal with the phosphates of the nucleotide alters its hardness–softness properties so that it is much closer to that of potential binding sites on the base thereby enhancing complexation with the ring [2]. There is some circumstantial experimental evidence favoring the first possibility. In the case of the Ni(II)–ATP complex a background complex has been shown to occur [4]. When ribosome of ATP is replaced by glucose the molecule cannot fold back on itself and Ni(II) bound to the three phosphate groups does not interact with the adenine ring [3]. However, since the molecule cannot fold back, no interaction would occur even if the second proposition were correct.

In order to test possibility (b) more extensively we have examined the ability of Ni(II) to interact with adenosine in the presence and absence of inorganic tripolyphosphate (PPP_i). Under these circumstances, where the triphosphate group is not attached to the nucleoside, possibility (a) does not exist. We have reported previously that the presence of PPP_i enhances the interaction of Ni(II) with adenosine as determined by spectrophotometric measurements [3]. This type of measurement provides no information regarding the site of interaction with the adenine ring. In order for possibility (b) to be correct, however, it is necessary to prove that the site of interaction is at N-7 as is the case in the Ni(II)–ATP complex. In the present work we used nmr to study the interaction of Ni(II) with adenosine in the presence and absence of PPP_i. Our results support the conclusion that the reason Ni(II) interacts with adenine in the nucleotide but not the nucleoside is that complexation with the phosphates alters its properties thereby facilitating reaction with the most favored site on the adenine ring.

2. Materials and methods

Solutions in D_2O of 0.08 M adenosine containing varying amounts of $Ni(NO_3)$ or $Ni(NO_3)_2$–PPP_i (1:1) were prepared as previously described [4]. The final pH was 5.5 ± 0.2. Measurements were made with a Varian HA-100 spectrometer with tetramethylammonium ion as an internal standard.

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3. Results and discussion

Fig. 1 shows that when Ni(II) is added to an aqueous solution of 0.08 M adenosine there is a small and equal effect on the chemical shift of the H-8 and H-2 protons. When triphosphate is added the chemical shift of both protons is increased but there

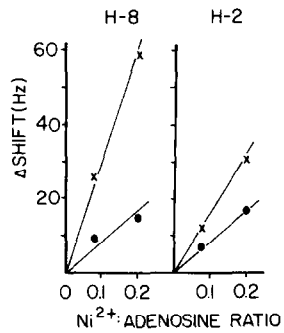

Fig. 1. Change in chemical shift of ring protons as function of Ni(II):adenosine ratio. (•–• no inorganic triphosphate; (x–x) contains inorganic triphosphate at same concentration as Ni(II). Adenosine = 0.08 M, pD = 5.5 ± 0.5.

is a much larger effect on the H-8 proton. When these changes in chemical shift are extrapolated to a Ni(II): adenosine ratio of one, the estimated shifts obtained with Ni(II)–PPP_i–adenosine are consistent with those of the outer sphere Ni(II)–ATP complex (table 1). It appears that inorganic triphosphate increases the ability of Ni(II) to bind to the adenine ring of adenosine and also increases the selectivity of the Ni(II) for the N-7 site.

These data clearly show that the interaction of Ni(II) with the ring of ATP arises primarily via the formation of a Ni(II)–PPP_i complex and not from the structural relationship of the adenine ring to the triphosphate group.

Table 1
Effect of inorganic triphosphate on the chemical shifts of Ni(II)–adenosine proton magnetic resonance signal

Compound	Change in chemical shift (Hz)	
	H-8	H-2
Ni(II)–ATP	510	160
Ni(II)–adenosine	80	80
Ni(II)–PPP_i–adenosine	300	150

The values shown were obtained by extrapolating experimental values to a Ni(II) to nucleotide or nucleoside ratio of one. The Ni(II)–ATP data comes from ref. [4].

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